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An Infant With a Mosaic 45,X/46,X,psu dic(Y) (pter→q11.2::q11.2→pter) Karyotype and Mixed Gonadal Dysgenesis Studied for Extent of Mosaicism in the Gonads

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An infant with mixed gonadal dysgenesis was found to have a 45,X/46,X,psu dic(Y) karyotype. A low level (8%) of mosaicism for the dic(Y) cell line was observed in peripheral blood lymphocytes and skin fibroblasts. The dicentric nature of the Y chromosome became apparent in fluorescence in situ hybridization studies. The presence of Y centromeric sequences was demonstrated in the paraffin-embedded testis and streak ovary sections. The ratio of Y-positive cells was higher in the testis than in the streak ovary.

KEY WORDS: 45,X/46,X,psu dic(Y); fluorescence in situ hybridization; mosaicism in blood, skin, testis, and streak ovary

INTRODUCTION

Most reported cases of dicentric Y are found as mosaics with a 45,X cell line [Alexander et al., 1978; Kaluzewski et al., 1988; Speleman et al., 1990; Fujimoto et al., 1991]. The clinical presentation of these patients ranges from phenotypic females with ovarian failures to males with dysgenetic gonads. A similar range of phenotype is also seen in Ullrich-Turner syndrome (UTS) mosaics with a 46,XY cell line. Therefore, variation in phenotype may be related to the degree of mosaicism. The detection of a Y cell line is important in the prospective management of patients with dysgenetic gonads, because they have an increased risk for gonadoblastoma.

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In this study, a patient with mixed gonadal dysgenesis was evaluated for mosaicism in the gonads to see if there was a genotype difference between the testis and streak ovary. The clinical and cytogenetic findings are discussed.

CASE REPORT

A premature newborn infant, the second twin, was investigated because of ambiguous genitalia. The first twin was a normal female. There was no known parental consanguinity. The family history was unremarkable. During pregnancy there was no evidence of intrauterine growth retardation. At 33 weeks gestation, birth weight was 2,183 g (75th centile), head circumference 31.75 cm (75th centile), and length 47.5 cm (90th centile). The external genitalia consisted of a 2.5-cm long phallus with chordee, bifid scrotum, hypospadias at the junction of phallus and scrotum, a palpable gonad in the right sac of the scrotum (resembling a normal-sized newborn testis), and an empty left sac. A small mass was palpable over the left inguinal region. Abdominal sonograms and voiding cystourethrogram showed a urogenital sinus, vagina, uterus, the left inguinal canal containing a fallopian tube, a small gonad, and a 2-cm diameter dysplastic complex cystic malformation at the upper pole of the right kidney. The endocrine study showed marked increase in serum testosterone after 3 days of administration of human chorionic gonadotropin. During surgery, the right gonad and the left streak ovary were biopsied. The left fallopian tube, streak ovary, and the remnants of a streak ovary on the right side were excised. Bilateral herniorrhaphy was performed. Hysterectomy and removal of vagina are planned for the future, as the patient is being reared as a boy. On histologic examination, the right gonad contained seminiferous tubules with spermatogonia and Sertoli cells. Leydig cells and epididymis were identified. An ovotestis was ruled out. The biopsy specimen from the left side showed germ cells with spindle stroma (consistent with streak ovary), fallopian tube, and remnant of Wolffian duct. There was no evidence of gonadoblastoma in any examined sections.

CYTOGENETIC STUDIES

The patients' peripheral blood was sent to Corning Nichols Institute for chromosome studies. Twenty-nine G-banded metaphases were 45,X and one was 46,XY. A normal size Y chromosome was observed. A fluorescence in situ hybridization (FISH) study using the Y centromeric alpha satellite probe, DYZ3 (Oncor), was performed according to the manufacturer's instructions. Two of 65 metaphases had a Y chromosome *with two signals* (Fig. 1), and 45 of 590 interphases (~8%) were positive for the Y with the signal appearing as two dots. A skin biopsy culture was set up. G-banded slides were analyzed, and 4 of 50 metaphases were 46,XY. In one of four metaphases, the Y appeared dicentric. In FISH preparations, 6 of 63 metaphases and 59 of 709 interphase nuclei (~8%) were positive with two Y centromeric signals. The Y heterochromatin region that fluoresces brightly with 4′, 6-diamidino-2-phenylindole (DAPI) was not observed. From these studies, it was concluded that this patient had a 45,X/46,X,psu dic(Y)(pter→q11.2::q11.2→pter) karyotype. The father's karyotype was normal, and the Y chromosome had a large qh region.

Paraffin-embedded tissue blocks of the streak ovary and testis were sectioned. Four-micron sections were taken and placed on slides treated with 3-aminopropyltriethoxysilane (Sigma). FISH using X and Y centromeric probes DXZ1 and DYZ3 was performed following the manufacturer's instructions (Oncor). Both streak ovary and testis had Y-containing cells (Fig. 2). The intratubular cells in the testis and the large germ cells in the streak ovary were scored for X and Y signal. The FISH analysis was performed as follows. First, the Y-alpha satellite probe was used, with 31 of 332 cells (~9%) in the ovary and 78 of 369 cells (~21%) in the testis being Y centromere positive. Second, the X- and Y-alpha satellite single color probes were used to see if the Y probe values were low because of hybridization inefficiency. In our experience with cytogenetic diagnostic specimens, the hybridization efficiency of the X and Y probes has been similar. Therefore, cells with hybridization signal(s) were scored. A single signal was assumed to represent a 45,X cell, although we cannot exclude the possibility that some were lacking an X chromosome or its hybridization signal. Using this approach (i.e., X- and Y-alpha satellite single color assay), 35 of 535 cells (~7%) in the ovary and 49 of 349 cells (~14%) in the testis were Y centromere positive. Finally, a repeat hybridization using the X- (P5060-FITC, Oncor) and Y- (P5065-DG.5, Oncor) alpha satellite dual color probes was performed to eliminate any scoring errors. In the X- and Y-alpha satellite dual color probe study, we analyzed 500 cells from the ovary and 490 cells from the testis in which a single X chromosome signal was present. Of these, 33 of 500 cells (~7%) in the ovary and 89 of 490 cells (~18%) in the testis were Y centromere positive. This represents a highly significant difference ($\chi^2 = 30.58$; $P < 0.001$). Based on these three different analyses, we conclude that the testis had approximately twice as many Y cells (14–21%) compared with the ovary (7–9%).

DISCUSSION

The dicentric Y is almost always present as a mosaic with a 45,X cell line. Our patient also has low-level mosaicism for the 46,X,psu dic(Y) cell line. The frequent

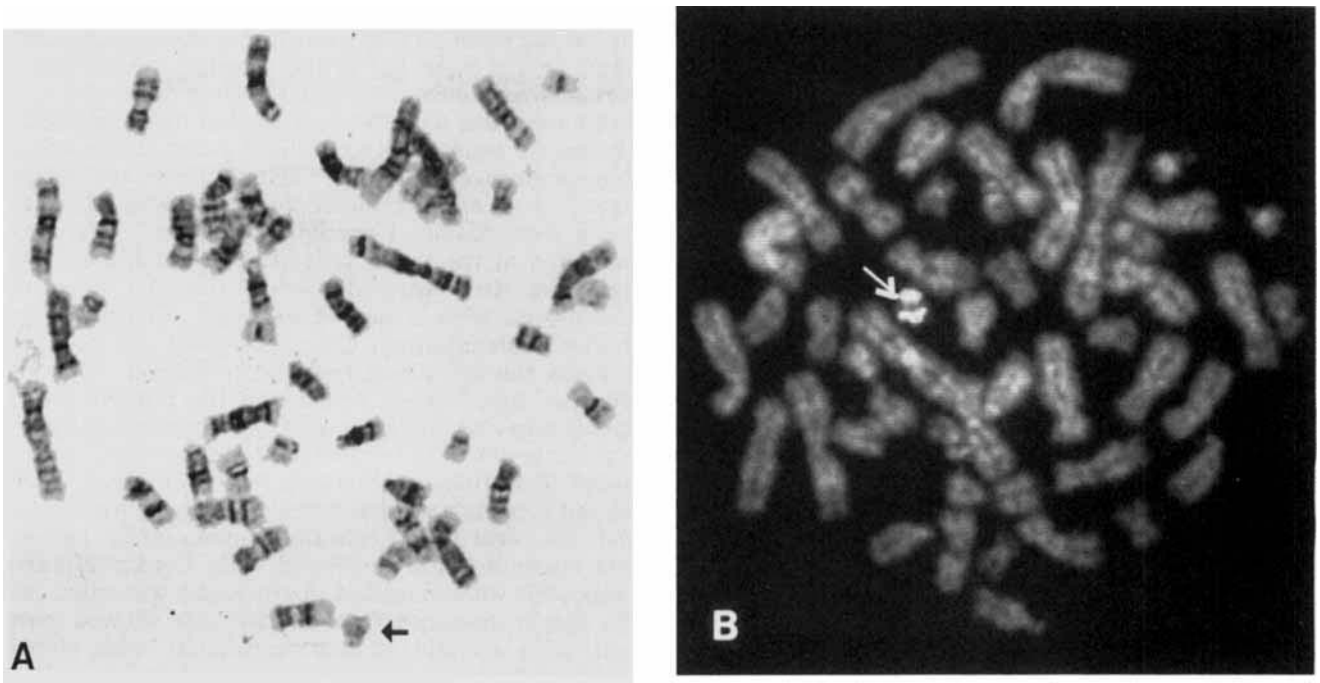

Fig. 1. **A:** G-banded psu dic (Y) chromosome. **B:** FISH using Y alpha satellite probe shows hybridization to two centromeres on the Y chromosome.

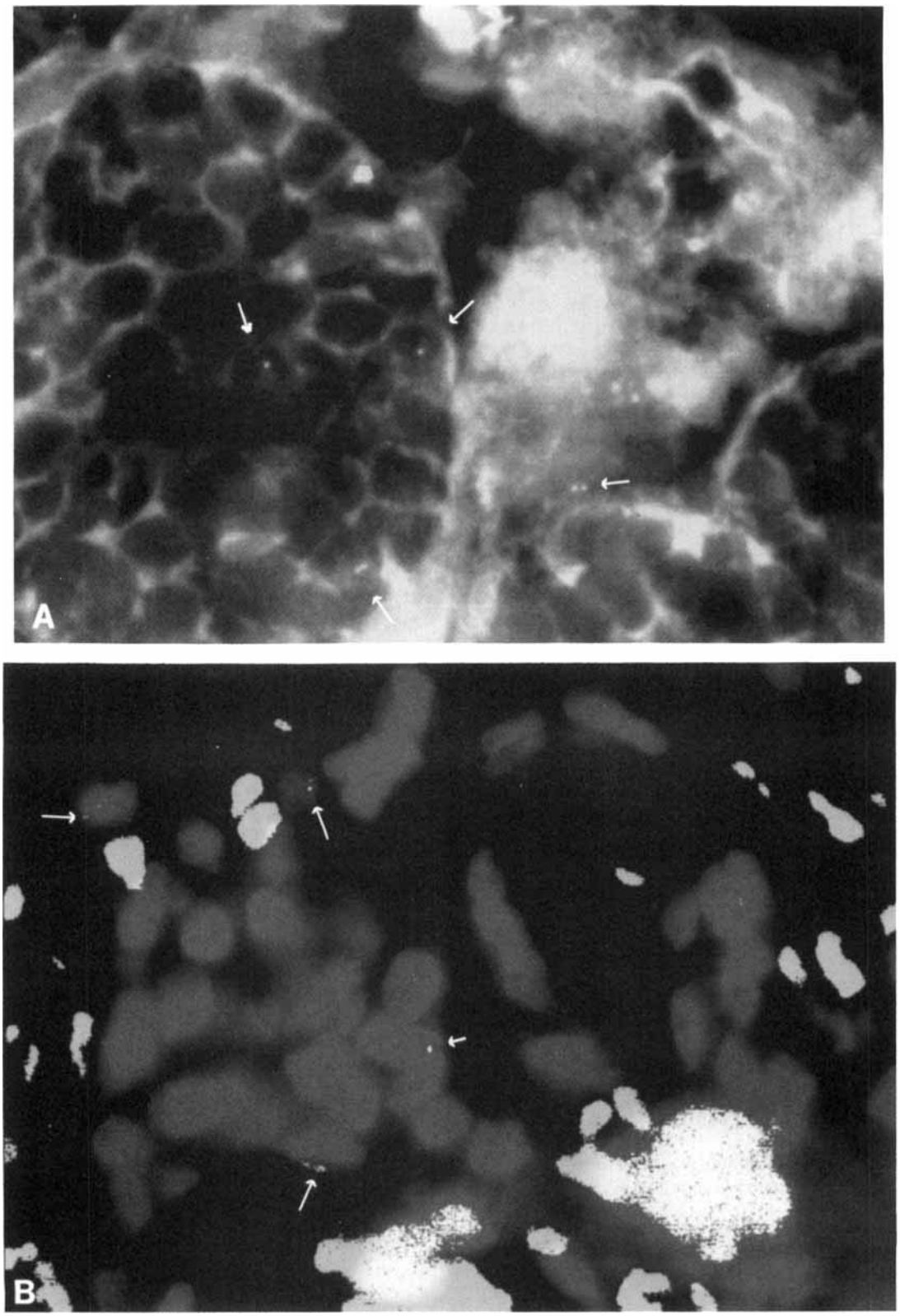

Fig. 2. **A:** Section of testes. Arrows indicate cells with hybridization signal(s) for the Y centromere probe. **B:** Section of streak ovary. Arrows show cells with hybridization signal(s) for the Y centromere probe.

loss of the Y is probably because it is dicentric and mitotically unstable. However, Held et al. [1992] suggest that there may be an in vivo selection against rearranged sex chromosomes, based on the continual decrease of the abnormal sex chromosome observed in in vitro long-term culture.

The Y chromosome in our study appeared to be of normal length and nonfluorescent. This morphology strongly indicates an isodicentric Y [Magenis and Donlon, 1982; Speleman et al.,1990]. In routine G-banded cells, it was hard to detect the dicentric nature of the Y chromosome, possibly because one of the centromeres was inactive; however, in FISH studies the Y centromeric probe consistently yielded two signals.

The phenotypic manifestations associated with a dicentric Y range from females with ovarian failures to males with dysgenetic gonads. The phenotypic range was best illustrated by the report of monozygotic twins with a 45,X/46,X,idic(Y) mosaicism [Fujimoto et al., 1991]. One twin was a girl with Ullrich-Turner syndrome, and the other was an apparently normal boy. The conclusion drawn from this and other studies is that the very different phenotypes are caused by the unequal distribution of the two cell lines in various tissues. Therefore, in our patient, we examined the extent of mosaicism in the testis and streak ovary to see if there was a difference in distribution of the two cell lines. The paraffin-embedded testis and ovary sections were probed with X and Y centromeric sequences. Scoring proved a challenge because of many overlapping cells, and only a gross estimate of mosaicism was possible. Both testis and streak ovary had Y-positive cells. However, the testis had significantly more Y-containing cells than the streak ovary. The percentage of cells with a Y chromosome in the ovary seems to coincide with that found in the blood and skin. We expected the Y chromosome to be present in the testis and absent in the ovary. Because the Y chromosome was found in both gonads, we postulated that the difference in ratio of Y-bearing cells observed in the testis and ovary may be the cause for divergent gonadal development.

In early murine and probably human development, the supporting cell lineage in the genital ridge is stimulated to differentiate into Sertoli cells by a key testis-determining gene on the Y chromosome called the SRY (sex-determining region on the Y) [Jeske et al.,1995]. The Sertoli cell differentiation results in other cell lineages such as steroid-producing cells, connective tissue cells, and germ cells to take the male pathway. The seminiferous tubules appear at 6 weeks of gestation. In the absence of the testis-determining gene, the ovarian route is followed.

In our patient, the following scenario of events in early gonadal differentiation can be envisaged. There were fewer Y-containing cells in the left streak ovary than in the right testis. Therefore, by extrapolation, the left gonadal ridge may have had negligible Y-containing supporting cells. Remnants of a streak ovary were also present on the right side. This could presumably reflect clustering of Y-positive supporting cells in the gonadal ridge. An area of the gonadal ridge may have lacked Y-containing supporting cells and, by default, gone on to become a streak ovary, whereas the area with Y-positive supporting cells probably developed into a testis.

Assessing mosaicism in many organs and in specific cell types has become possible with the introduction of the FISH technique. However, associating the observed mosaicism to a phenotype may be too simplistic because it does not reflect the embryonic distribution of the two clones in the different embryonic cell lineages, nor does it take into account the complex dynamics of early development.

The presence of a cell line with a Y chromosome in a 45,X mosaic patient increases the risk for gonadoblastoma. Detection of the Y chromosome in the gonads further ratifies the need for surveillance for gonadal tumors. Therefore, in our proband, the streak ovaries were surgically removed and the morphologically normal testis was retained.

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